

5 What is claimed is:

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1. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

10 (a) collecting a plasma sample from the HIV-infected patient;

(b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 88; and

15 (c) determining increased susceptibility to amprenavir.

2. The method of claim 1, wherein the mutation at codon 88 codes for a serine (S).

20 3. The method of claim 1, wherein the HIV-infected patient is being treated with an antiretroviral agent.

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25 4. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

(a) collecting a plasma sample from the HIV-infected patient;

(b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 88 and additional mutations at codons 63 and/or 77 or a combination thereof; and

(c) determining decreased susceptibilty to nelfinavir

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- 5 9. The method of claim 7, wherein the HIV-infected patient
is being treated with an antiretroviral agent.

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10. A method of assessing the effectiveness of protease
antiretroviral therapy of an HIV-infected patient

10 comprising:

(a) collecting a plasma sample from the HIV-infected
patient;

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15 (b) evaluating whether the plasma sample contains
nucleic acid encoding HIV protease having a mutation at
codon 88 and additional mutations at codons 63, 77, 46,
10, 20, and/or 36 or a combination thereof; and

(c) determining decreased susceptibility to nelfinavir
and indinavir and increased susceptibility to
amprenavir.

- 20 11. The method of claim 10, wherein the mutation at codon
63 codes for a proline (P) or a glutamine (Q), the
mutation at codon 77 codes for an isoleucine (I), the
mutation at codon 46 codes for a leucine (L) or an
25 isoleucine (I), the mutation at codon 10 codes for a
isoleucine (I) or a phenylalanine (F), the mutation at
20 codes for a threonine (T) or a methionine (M) or an
arginine (R), and the mutation at 36 codes for an
isoleucine (I) or a valine (V).

- 30 12. The method of claim 10, wherein the HIV-infected
patient is being treated with an antiretroviral agent.

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5 13. A method for evaluating the biological effectiveness of
a candidate HIV antiretroviral drug compound
comprising:

- 10 (a) introducing a resistance test vector comprising a
patient-derived segment further comprising a mutation
at codon 88 and an indicator gene into a host cell;
(b) culturing the host cell from step (a);
(c) measuring the indicator in a target host cell; and
15 (d) comparing the measurement of the indicator from
step (c) with the measurement of the indicator measured
when steps (a) - (c) are carried out in the absence of
the candidate antiretroviral drug compound;
wherein a test concentration of the candidate
antiretroviral drug compound is present at steps (a) -
20 (c); at steps (b) - (c); or at step (c).

20 14. A method for evaluating the biological effectiveness of
a candidate HIV antiretroviral drug compound
comprising:

- 25 (a) introducing a resistance test vector comprising a
patient-derived segment further comprising a mutation
at codon 88 and mutation(s) at codons 63 and/or 77 or a
combination thereof and an indicator gene into a host
cell;
(b) culturing the host cell from step (a);
30 (c) measuring the indicator in a target host cell; and
(d) comparing the measurement of the indicator from
step (c) with the measurement of the indicator measured
when steps (a) - (c) are carried out in the absence of

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5 the candidate antiretroviral drug compound;
wherein a test concentration of the candidate
antiretroviral drug compound is present at steps (a) -
(c); at steps (b) - (c); or at step (c).

10 15. A method for evaluating the biological effectiveness of
a candidate HIV antiretroviral drug compound
comprising:

15 (a) introducing a resistance test vector comprising a
patient-derived segment further comprising a mutation
at codon 88 and mutation(s) at codons 63, 77, and/or 46
or a combination thereof and an indicator gene into a
host cell;

20 (b) culturing the host cell from step (a);
(c) measuring the indicator in a target host cell; and
(d) comparing the measurement of the indicator from
step (c) with the measurement of the indicator measured
when steps (a) - (c) are carried out in the absence of
25 the candidate antiretroviral drug compound;

wherein a test concentration of the candidate
antiretroviral drug compound is present at steps (a) -
(c); at steps (b) - (c); or at step (c).

30 16. A method for evaluating the biological effectiveness of
a candidate HIV antiretroviral drug compound
comprising:

(a) introducing a resistance test vector comprising a

- 5 patient-derived segment further comprising a mutation at codon 88 and mutation(s) at codons 63, 77, 46, 10, 20, and/or 36 or a combination thereof and an indicator gene into a host cell;
- (b) culturing the host cell from step (a);
- 10 (c) measuring the indicator in a target host cell; and
- (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a) - (c) are carried out in the absence of the candidate antiretroviral drug compound;
- 15 wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a) - (c); at steps (b) - (c); or at step (c).
17. A resistance test vector comprising an HIV —
- 20 patient-derived segment further comprising protease having a mutation at codon 88 and an indicator gene, wherein the expression of the indicator gene is dependent upon the patient derived segment.
- 25 18. The resistance test vector of claim 17, wherein the patient-derived segment having a mutation at codon 88 further comprises mutations at codons 63 and 77 or a combination thereof.
- 30 19. The resistance test vector of claim 17, wherein the patient-derived segment having a mutation at codon 88 further comprises mutations at codons 63, 77 and/or 46 or a combination thereof.

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20. The resistance test vector of claim 17, wherein the patient-derived segment having a mutation at codon 88 further comprises mutations at codons 63, 77, 46, 10, 20 and/or 36 or a combination thereof.

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21. A method for evaluating the viral fitness of a patient's virus comprising:

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(a) introducing a resistance test vector comprising a patient-derived segment from a patient's virus and an indicator gene into a host cell;

(b) culturing the host cell from step (a);

(c) measuring the luciferase activity in a target host cell in the absence of any antiretroviral drug; and

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(d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a)-(c) are carried out for a reference control in the absence of any antiretroviral drug;

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wherein a reduction in the luciferase activity measured in step (c) as compared to step (d) indicates a reduction in viral fitness.

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22. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

(a) collecting a plasma sample from the HIV-infected patient;

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(b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 82 and an additional mutation at codon 24; and

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27. The method of claim 25, wherein the HIV-infected

5 patient is being treated with an antiretroviral agent.

28. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

(a) collecting a plasma sample from the HIV-infected patient;

(b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 82 and an additional mutation at codon 71; and

(c) determining decreased susceptibility to indinavir.

29. The method of claim 28, wherein the mutation at codon 71 codes for an amino acid selected from the group consisting of a threonine, (T) valine, (V) leucine (L) and isoleucine (I).

30. The method of claim 28, wherein the HIV-infected patient is being treated with an antiretroviral agent.

31. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

(a) collecting a plasma sample from the HIV-infected patient;

(b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at

5 codon 82 and additional mutations at codons selected from the group consisting of codon 54, 46, 10, 63, and a combination thereof; and
(c) determining decreased susceptibility to indinavir.

10 32. The method of claim 31, wherein the mutation at codon 54 codes for an amino acid selected from the group consisting of a valine (V), alanine (A), leucine (L) and threonine (T), the mutation at codon 46 codes for an amino acid selected from the group consisting of a
15 leucine (L), isoleucine (I) and valine (V), the mutation at codon 10 codes for an amino acid selected from the group consisting of an isoleucine (I), valine (V), phenylalanine (F), and arginine (R), and the mutation at codon 63 codes for an amino acid selected
20 from the group consisting of proline (P), alanine (A), serine (S), threonine (T), glutamine (Q), , cysteine (C), and valine (V).

25 33. The method of claim 31, wherein the HIV-infected patient is being treated with an antiretroviral agent.

30 34. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:
(a) collecting a plasma sample from the HIV-infected patient;
(b) evaluating whether the plasma sample contains

5 nucleic acid encoding HIV protease having a mutation at
codon 82 and an additional mutation at codon 20; and
(c) determining decreased susceptibility to saquinavir.

10 35. The method of claim 34, wherein the mutation at codon
20 codes for an amino acid selected from the group
consisting of a methionine (M), threonine (T),
isoleucine (I), and arginine (R).

15 36. The method of claim 34, wherein the HIV-infected
patient is being treated with an antiretroviral
agent.

20 ✓ 37. A method of assessing the effectiveness of protease
antiretroviral therapy of an HIV-infected patient
comprising:
(a) collecting a plasma sample from the HIV-infected
patient;
(b) evaluating whether the plasma sample contains
nucleic acid encoding HIV protease having a mutation at
25 codon 82 and an additional mutation at codon 36; and
(c) determining decreased susceptibility to saquinavir.

30 38. The method of claim 37, wherein the mutation at codon
36 for an amino acid selected from the group consisting
of a isoleucine (I), leucine (L), and valine (V).

39. The method of claim 37, wherein the HIV-infected
patient is being treated with an antiretroviral

5 agent.

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40. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

10 (a) collecting a plasma sample from the HIV-infected patient;

(b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 82 and additional mutations at codons 24, 71, 54, and/or 10 or a combination thereof; and

15 (c) determining decreased susceptibility to saquinavir.

41. The method of claim 40, wherein the mutation at codon 24 codes for an isoleucine (I), the mutation at codon 71 codes for an amino acid selected from the group consisting of a threonine (T), valine (V), leucine (L), and isoleucine (I), the mutation at codon 54 codes for an amino acid selected from the group consisting of valine (V), alanine (A), leucine (L), and threonine (T), and the mutation at codon 10 codes for an amino acid selected from the group consisting of an isoleucine (I), valine (V), phenylalanine (F), and arginine (R).

30 42. The method of claim 40, wherein the HIV-infected patient is being treated with an antiretroviral agent.

5 43. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

(a) collecting a plasma sample from the HIV-infected patient;

10 (b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 82 and the number of additional mutations at secondary positions; and

15 (c) determining decreased susceptibility to indinavir and saquinavir.

44. The method of claim 43, wherein the number of additional mutations at secondary positions is at least 3.

20 45. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

25 (a) collecting a plasma sample from the HIV-infected patient;

(b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 90 and secondary mutations; and

30 (c) determining changes in susceptibility to ritonavir, nelfinavir, indinavir, saquinavir and amprenavir.

46. The method of claim 45, wherein the mutation at codon

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5 90 codes for a methionine.

47. The method of claim 45, wherein the HIV-infected patient is being treated with an antiretroviral agent.

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48. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

15 (a) collecting a plasma sample from the HIV-infected patient;

(b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 90 and an additional mutation at codon 73; and

20 (c) determining decreased susceptibility to indinavir.

49. The method of claim 48, wherein the mutation at codon 73 codes for an amino acid selected from the group consisting of a serine (S), threonine (T), and cysteine (C).

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50. The method of claim 48, wherein the HIV-infected patient is being treated with an antiretroviral agent.

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51. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

- 5 (a) collecting a plasma sample from the HIV-infected patient;
- (b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 90 and an additional mutation at codon 71; and
- 10 (c) determining decreased susceptibility to indinavir.
52. The method of claim 51, wherein the mutation at codon 71 codes for an amino acid selected from the group consisting of a threonine (T), valine (V), leucine (L), and isoleucine (I).
- 15 53. The method of claim 51, wherein the HIV-infected patient is being treated with an antiretroviral agent.
- 20 54. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:
- (a) collecting a plasma sample from the HIV-infected patient;
- 25 (b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 90 and an additional mutation at codon 46;; and
- (c). determining decreased susceptibility to indinavir.
- 30 55. The method of claim 54, wherein the mutation at codon 46 codes for an amino acid selected from the group consisting of a leucine (L), isoleucine (I) and valine (V).

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56. The method of claim 54, wherein the HIV-infected patient is being treated with an antiretroviral agent.

10 57. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

(a) collecting a plasma sample from the HIV-infected patient;

15 (b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 90 and an additional mutation at codon 73; and

(c) determining decreased susceptibility to saquinavir.

20 58. The method of claim 57, wherein the mutation at codon 73 codes for an amino acid selected from the group consisting of a serine (S), threonine (T), and cysteine (C).

25 59. The method of claim 57, wherein the HIV-infected patient is being treated with an antiretroviral agent.

30 60. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

(a) collecting a plasma sample from the HIV-infected patient;

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- 5 (b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 90 and an additional mutation at codon 71; and
 (c) determining decreased susceptibility to saquinavir.

10 61. The method of claim 60, wherein the mutation at codon 71 codes for an amino acid selected from the group consisting of a threonine (T), valine (V), leucine (L), and isoleucine (I).

15 62. The method of claim 60, wherein the HIV-infected patient is being treated with an antiretroviral agent.

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20 63. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

- (a) collecting a plasma sample from the HIV-infected patient;
 (b) evaluating whether the plasma sample contains
25 nucleic acid encoding HIV protease having a mutation at codon 90 and additional mutations at codons 77 and 10; and
 (c) determining decreased susceptibility to saquinavir.

30 64. The method of claim 63, wherein the mutation at codon 77 codes for an amino acid selected from the group consisting of isoleucine (I) and threonine (T) and the mutation at codon 10 codes for an amino acid selected

5 from the group consisting of isoleucine (I), valine
(V), phenylalanine (F), and arginine (R).

65. The method of claim 63, wherein the HIV-infected
patient is being treated with an antiretroviral
10 agent.

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66. A method of assessing the effectiveness of protease
antiretroviral therapy of an HIV-infected patient
comprising:
15 (a) collecting a plasma sample from the HIV-infected
patient;
(b) evaluating whether the plasma sample contains
nucleic acid encoding HIV protease having a mutation at
codon 90 and the number of additional mutations at
20 secondary positions; and
(c) determining decreased susceptibility to indinavir
and saquinavir.

67. The method of claim 66, wherein the number of
25 additional mutations at secondary positions is at least
3.

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68. A method of assessing the effectiveness of protease
antiretroviral therapy of an HIV-infected patient
comprising:
30 (a) collecting a plasma sample from the HIV-infected
patient;
(b) evaluating whether the plasma sample contains

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(c) measuring the indicator in a target host cell; and
(d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a) - (c) are carried out in the absence of

5 the candidate antiretroviral drug compound;

wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a) - (c); at steps (b) - (c); or at step (c).

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✓ 72. A method for evaluating the biological effectiveness of a candidate HIV protease antiretroviral drug compound comprising:

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(a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 82 and secondary mutation(s) at codons 20, 24, 71, 54 and/or 10 or a combination thereof and an indicator gene into a host cell;

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(b) culturing the host cell from step (a);
(c) measuring the indicator in a target host cell; and
(d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a) - (c) are carried out in the absence of the candidate antiretroviral drug compound;

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wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a) - (c); at steps (b) - (c); or at step (c).

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✓ 73. A method for evaluating the biological effectiveness of a candidate HIV protease antiretroviral drug compound comprising:

(a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation

5 at codon 90 and additional mutations at one or more
secondary positions and an indicator gene into a host
cell;

(b) culturing the host cell from step (a);

(c) measuring the indicator in a target host cell; and

10 (d) comparing the measurement of the indicator from
step (c) with the measurement of the indicator measured
when steps (a) - (c) are carried out in the absence of
the candidate antiretroviral drug compound;

15 wherein a test concentration of the candidate
antiretroviral drug compound is present at steps (a) -
(c); at steps (b) - (c); or at step (c).

20 74. A method for evaluating the biological effectiveness of
a candidate HIV protease antiretroviral drug compound
comprising:

(a) introducing a resistance test vector comprising a
patient-derived segment further comprising a mutation
25 at codon 90 and secondary mutation(s) at codons 73, 71,
10 and/or 46 or a combination thereof and an indicator
gene into a host cell;

(b) culturing the host cell from step (a);

(c) measuring the indicator in a target host cell; and

30 (d) comparing the measurement of the indicator from
step (c) with the measurement of the indicator measured
when steps (a) - (c) are carried out in the absence of
the candidate antiretroviral drug compound;

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wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a) - (c); at steps (b) - (c); or at step (c).

10 75. A method for evaluating the biological effectiveness of a candidate HIV protease antiretroviral drug compound comprising:

15 (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codons 82 and 90 and additional mutations at one or more secondary positions and an indicator gene into a host cell;

(b) culturing the host cell from step (a);

(c) measuring the indicator in a target host cell; and

20 (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a) - (c) are carried out in the absence of the candidate antiretroviral drug compound;

25 wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a) - (c); at steps (b) - (c); or at step (c).

30 76. A resistance test vector comprising an HIV patient-derived segment further comprising protease having a mutation at codon 82 and an indicator gene, wherein the expression of the indicator gene is dependent upon the patient derived segment.

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77. The resistance test vector of claim 76, wherein the patient-derived segment having a mutation at codon 82 further comprises at least one secondary mutation at a codon selected from the group consisting of 20, 24, 71, 54, 10 and a combination thereof.

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78. The resistance test vector of claim 76, wherein the patient-derived segment having a mutation at codon 90 further comprises at least one secondary mutation at a codon selected from the group consisting of 73, 71, 46, 10 and a combination thereof.

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79. A method for determining replication capacity for a patient's virus comprising:

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(a) introducing a resistance test vector comprising a patient derived segment and an indicator gene into a host cell;

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(b) culturing the host cell from (a);

(c) harvesting viral particles from step (b) and infecting target host cells;

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(d) measuring expression of the indicator gene in the target host cell, wherein the expression of the indicator gene is dependent upon the patient-derived segment;

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